T lymphocyte subpopulations diverge in commercially raised chickens

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Abstract

To evaluate immunocompetence in commercially raised chickens, we immunophenotyped Dekalb Delta and H&N White Leghorn (WLH) hybrids, 20 chickens in each of 3 age groups (9 wk [juvenile], 25 wk [young adult], and 79 or 80 wk [adult]), for circulating $CD3^+$, $CD4^+$, $CD8^+$, $TCR1^+$, $TCR2^+$, and $TCR3^+$ lymphocytes. The proportion of $CD3^+$ T cells, including $CD4^+$ and $CD8^+$ subsets, was increased in the hybrids as compared with published values for laboratory-raised outbred WLH chickens. The proportion of the $TCR2^+$ (Vβ1) T cell subpopulation was also increased. An age-related decrease in the proportion of $TCR1^+$ (γδ) T cells was noted in both hybrids. Further, a remarkably low CD4:CD8 ratio was evident in all age groups of both hybrids, indicating decreased immunocompetence. Overall, these experiments provide age-related proportions of various peripheral-blood T lymphocyte subpopulations in commercially raised Dekalb Delta and H&N chickens that diverge from the proportions in laboratory-raised outbred WLH chickens and suggest reduced immunocompetence. Such a decline in immunocompetence, including humoral immune capacity, could be attributed to genetic selection for production traits, environmental factors associated with commercial operations, and intense immunization.

Résumé

L'immunophénotypage de poulets hybrides Dekalb Delta et H&N White Leghorn (WLH) a été effectué afin d'évaluer l'immunocompétence. Pour chacun des groupes d'âge étudiés (9 sem [juvénile], 25 sem [jeune adulte] et 79 ou 80 sem [adulte]), on a typé chez 20 poulets élevés commercialement les lymphocytes circulants $CD3^+$, $CD4^+$, $CD8^+$, $TCR1^+$, $TCR2^+$ et $TCR3^+$. Les proportions de cellules T $CD3^+$, incluant les sous-groupes $CD4^+$ et $CD8^+$, étaient augmentées chez les hybrides lorsque comparées avec des données publiées pour des poulets non-cosanguins WLH élevés en laboratoire. La proportion de la sous-population de cellules T $TCR2^+$ (Vβ1) était également augmentée. Une diminution en fonction de l'âge pour la proportion de cellules T $TCR1^+$ (γδ) fût également notée pour les deux hybrides. De plus, un ratio remarquablement bas CD4:CD8 était évident dans tous les groupes d'âge des deux hybrides, indiquant ainsi une diminution de l'immunocompétence. Globalement, ces expériences fournissent, pour des poulets Dekalb Delta et H&N élevés commercialement, des proportions pour diverses sous-populations de lymphocytes T périphériques en fonction de l'âge qui divergent des proportions obtenues chez des poulets non-cosanguins WLH élevés en laboratoire et qui suggèrent une réduction de l'immunocompétence. Une telle réduction de l'immunocompétence, incluant les capacités de l'immunité humorale, pourrait être attribuée à la sélection génétique pour des caractères de production, à des facteurs environnementaux associés aux opérations commerciales et à une immunisation intensive.

(Traduit par Docteur Serge Messier)

Introduction

Genetically determined immunocompetence and environmental factors are responsible for varying susceptibility or resistance to infectious disease across species, with economically important chickens no exception (1). Studies of the immune system of chicken species have advanced our understanding of fundamental immunologic processes and principles (2), apart from demonstrating limited germline diversity (1), which is of direct relevance to immune functions responsible for host defence. Commercially raised chickens also have diminished polymorphism at loci of the major histocompatibility complex (3) that are known to be associated with disease resistance or susceptibility (1,4,5).

The immune competence of a host can be evaluated from several parameters, including circulating T lymphocyte populations (6,7). Peripheral blood lymphocyte populations in mice (8), humans (9), and chickens (1,10,11) have been suggested to be under genetic control. Further studies in mice and humans have demonstrated a decline in the function of the immune system with age (12), resulting in an increased incidence of both infectious and noninfectious diseases and increased mortality rates (13). The amount and proportion of T cell subsets have been correlated with disease susceptibility as well (1,14,15). Therefore, understanding age-related immunocompetence by evaluating circulating T lymphocyte populations in apparently healthy commercially raised chickens is of direct relevance to developing breeding strategies as well as promoting flock health measures.

As in mammals, the main effector cells in chickens are $CD3^+$ αβ TCR^+ T cells (16). But γδ TCR^+ T cells are a major circulating T cell subset in chickens, unlike mice and humans, and are identified by

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Table I. Vaccination protocols for commercially raised Dekalb Delta and H&N hybrids of White Leghorn (WLH) chickens

Dekalb Delta hybrid			H&N hybrid		
Week	Vaccine source	Organism (strain)	Week	Vaccine source	Organism (strain)
1 (hatch)	Marexine-SB (Intervet)	*Marek's disease virus* (HVT + SB1)	1 (hatch)	Marek's disease vaccine (select)	*Marek's disease virus* (HTV)
2	Clonevac-D78 (Intervet)	Infectious bursal disease (intermediate)	2	Bursine 2 (Solvay)	Infectious bursal disease (intermediate)
2	Duovac-Ma5 (Intervet)	Newcastle (B1) bronchitis (Mass-Conn)	2	Triplevac (Intervet)	Newcastle (B1) bronchitis (Mass-Conn)
4	Clonevac-D78 (Intervet)	Infectious bursal disease (intermediate)	4	Bursine 2 (Solvay)	Infectious bursal disease (intermediate)
4	Duovac-Ma5 (Intervet)	Newcastle (B1) bronchitis (Mass-Conn)			
6	Combovac-30 (Intervet)	Newcastle (LaSota) bronchitis (Mass-Conn)	6	Combovac-30 (Intervet)	Newcastle (LaSota) bronchitis (Mass-Conn)
7	Tremvac FP (Intervet)	Pox (avian fowl) avian encephalomyelitis (Calnek)	10	Tremvac FP (Intervet)	Pox (avian fowl) avian encephalomyelitis
7	Trachine (Intervet)	Laryngotracheitis	10	Laryngo-Vac (Solvay)	Laryngotracheitis
12	AvaBron HN63	Newcastle (LaSota) bronchitis (Mass)	12	Combovac-30 (Intervet)	Newcastle (LaSota) bronchitis (Mass-Conn)
			16	Combovac-30 (Intervet)	Newcastle (LaSota) bronchitis (Mass-Conn)

expression of TCR1 (17). Different subsets of αβ TcR^+ expressing either Vβ1 (TCR2) or Vβ2 (TCR3) or accessory molecules CD4 or CD8 (17,18) are known to have distinct functions.

Several studies have evaluated lymphocyte populations in the context of a particular disease (14,15,19,20), but little is known about age-related immunocompetence in healthy commercially raised chicken crosses with their individual management programs, including specific immunization protocols. To this end, we immunophenotyped peripheral blood lymphocytes from commercially raised juvenile, young-adult, and adult layer Dekalb Delta and H&N hybrids of White Leghorn (WLH) chickens for various T lymphocyte markers, including CD3 (21), CD4, CD8 (22), TCR1 (23), TCR2 (24), and TCR3 (21), and compared the results with published reference values (21–25) for unimmunized laboratory-raised outbred WLH chickens. We also compared the $CD4^+$:$CD8^+$ cell ratio, a measure of immunocompetence, in the hybrids and outbred WLH chickens (25).

Materials and methods

Chickens

Clinically healthy WLH layer chicken hybrids, Dekalb Delta (Dekalb Poultry Research, Dekalb, Illinois, USA) and H&N (H&N International, Redmond, Washington, USA), were obtained from poultry farms in Ontario. We included 20 chickens in each of 3 age groups — juvenile (9 wk old), young adult (25 wk old), and adult (80 wk old [Dekalb Delta] or 79 wk old [H&N]) — except for the juvenile Dekalb Delta, which numbered 19. The chickens were raised according to the breeders' management guides (26,27), including their immunization protocols (Table I), which differed between the 2 hybrids. The flocks from which the birds were taken had no history of disease and had a mortality rate lower than expected. The chickens were group housed on pine shavings in the Isolation Unit at the Ontario Veterinary College, University of Guelph, Guelph, Ontario. The housing area was scrubbed and steam-cleaned before the birds' arrival. Lighting and ventilation were identical for all the chickens. All birds were fed standard layer rations ad libitum. The experiments were performed according to the animal care guidelines of the Canadian Council on Animal Care to ensure humane handling and professional flock management.

Blood mononuclear cells

Heparinized blood collected from the chickens via jugular venipuncture was centrifuged (65 × *g*) at 20°C for 5 min, and the buffy coat was collected. The total number of lymphocytes was calculated in a Neubauer counting chamber (Hausser Scientific, Blue Bell, Pennsylvania, USA). The cell viability was consistently greater than 95% according to the trypan blue dye exclusion method.

Antibodies and reagents

Murine monoclonal antibodies specific for chicken CD3 (clone CT-3) and TCR3 (clone TCR3), both coupled to biotin, as well as CD4 (clone CT-4), CD8 (clone CT-8), TCR1 (clone TCR1), and TCR2 (clone TCR2), all coupled to fluorescein isothiocyanate (FITC; Southern Biotechnology Associates, Birmingham, Alabama, USA),

Table II. Proportions of peripheral blood T lymphocyte subsets in the 3 age groups of the 2 hybrids, as compared with published values for laboratory-raised outbred WLH chickens (25)

	Mean percentage of positive cells ± standard deviation (and range)						
		Dekalb Delta hybrid			H&N hybrid		
Surface molecule	Outbred WLH chickens	Juvenile[a] (*n* = 19)	Young adult[b] (*n* = 20)	Adult[c] (*n* = 20)	Juvenile[a] (*n* = 20)	Young adult[b] (*n* = 20)	Adult[d] (*n* = 20)
CD3	78 ± 3	93.7 ± 3.8 (83.6–97.9)	85.6 ± 7.9 (69.9–96.4)	88.5 ± 5.3 78.4–96.1	93.7 ± 4.0 79.2–97.5	90.2 ± 6.0 73.7–98.2	81.2 ± 7.1 68.9–92.9
CD4	39 ± 6	53.3 ± 5.7 (41.3–60.7)	43.1 ± 6.5 (30.8–55.8)	62.0 ± 7.8 (46.2–74.2)	58.7 ± 6.1 (44.2–68.8)	51.8 ± 10.1 (34.7–72.9)	56.0 ± 9.7 (40.8–75.4)
CD8	12 ± 2	44.1 ± 7.6 (34.6–65.1)	35.9 ± 8.7 (22.4–53.2)	35.7 ± 9.2 (19.5–52.6)	42.4 ± 8.6 (31.4–58.4)	40.8 ± 9.1 (27.3–56.2)	31.3 ± 7.2 (20.5–47.2)
$CD8^{dim}$	—	24.7 ± 6.8 (16.3–44.0)	22.0 ± 7.1 (12.1–40.7)	22.8 ± 8.9 (6.6–33.4)	32.6 ± 9.6 (16.6–49.6)	30.7 ± 11.2 (16.6–52.1)	25.3 ± 7.8 (15.2–44.9)
$CD8^{bright}$	—	19.4 ± 2.6 (15.7–24.1)	13.9 ± 5.0 (6.7–23.8)	13.0 ± 4.8 (4.4–22.4)	9.9 ± 3.0 (4.9–14.8)	10.0 ± 4.8 (3.5–21.6)	6.0 ± 4.0 (0.9–14.3)
$CD8^{dim}$:$CD8^{bright}$ ratio	—	1.3 ± 0.4 (0.8–2.1)	1.8 ± 1.0 (0.8–4.3)	2.2 ± 1.6 (0.5–6.6)	3.8 ± 1.9 (1.1–8.7)	4.4 ± 3.7 (0.8–12.9)	7.7 ± 8.2 (1.1–36.4)
TCR1	23 ± 3	22.9 ± 5.3 (16.2–33.2)	28.9 ± 7.7 (17.3–45.3)	13.8 ± 3.6 (8.7–21.6)	28.1 ± 4.1 (21.7–38.4)	28.3 ± 3.8 (22.7–34.1)	19.7 ± 7.3 (6.9–36.9)
TCR2	45 ± 2	54.1 ± 6.2 (42.5–64.6)	43.9 ± 8.1 (26.7–59.4)	56.1 ± 5.6 (45.8–65.5)	57.1 ± 5.1 (45.7–64.6)	50.5 ± 7.1 (36.1–63.3)	51.0 ± 7.1 (39.7–64.6)
TCR3	13 ± 2	17.0 ± 4.5 (8.6–27.7)	12.6 ± 4.0 (4.0–19.0)	18.8 ± 4.9 (10.5–28.0)	10.9 ± 1.1 (8.3–12.4)	10.7 ± 1.7 (7.0–13.5)	11.3 ± 3.0 (7.9–19.2)
CD4:CD8 ratio	3.25 ± 0.05	1.2 ± 0.2 (0.9–1.8)	1.3 ± 0.3 (0.7–1.9)	1.8 ± 0.5 (1.2–3.0)	1.4 ± 0.3 (1.1–2.2)	1.3 ± 0.3 (1.0–2.1)	1.9 ± 0.6 (1.0–3.3)

[a] Aged 9 wk
[b] Aged 25 wk
[c] Aged 80 wk
[d] Aged 79 wk

were used to detect chicken lymphocyte surface molecules. All antibodies were of IgG1κ isotype and were used at a concentration of 1 μg/mL. Neutralite avidin coupled to FITC (Southern Biotechnology Associates) was used as a secondary reagent. Isotype controls included mouse IgG1 coupled to FITC (Southern Biotechnology Associates) and biotinylated mouse IgG1, prepared by resuspending murine IgG1 (Chemicon International, Temecula, California, USA) in 100 μL of 0.06 M bicarbonate buffer (pH 8.5) at a concentration of 5 mg/mL with 50 μg of the biotinylating reagent (biotin [long arm] *N*-hydroxysuccinimidyl-6-hexanoate; Dimension Labs, Mississauga, Ontario), incubating the suspension at 20°C for 2 h, adding 10 μg of glycine, and dialyzing the mixture against phosphate-buffered saline (PBS), pH 7.4. Successful biotinylation was tested by labeling mouse splenocytes that bind IgG1 via the F_c receptor (28).

Flow cytometry

For single-color immunostaining, 1 million lymphocytes were incubated with specific antibodies (1 μg/mL) at 4°C for 30 min and then washed with PBS (pH 7.4) and 0.01% sodium azide. The cells incubated with biotinylated primary antibodies were stained with FITC-conjugated avidin, washed, and analyzed by flow cytometry (Becton-Dickinson Immunocytometry Systems, San Jose, California, USA) after resuspension in PBS containing propidium iodide (50 μg/mL). The lymphocytes were gated by forward scatter (FSC) and side scatter (SSC). Data were collected on 10 000 cells, converted to IBM-compatible format with HP Reader software (Becton-Dickinson, Mississauga, Ontario), and analyzed with WinMDI (Windows Multiple Document Interface) freeware.

For 2-color immunostaining, lymphocytes from five 19-wk-old H&N chickens were labeled with antibodies specific for CD3, CD4, and CD8 antigens together with isotype-matched controls. The labeled cells were analyzed by flow cytometry and the data displayed as 2-dimension plots of FITC (X-axis for CD4 or CD8) and phycoerythrin (Y-axis for CD3) fluorochromes.

Statistical analysis

The mean percentage, range, and standard deviation for each data set were calculated with the use of Statistix 7.0 software (Analytical Software, Tallahassee, Florida, USA). Statistically significant differences ($P \leq 0.05$) between means were tested by means of Kruskal–Wallis 1-way nonparametric analysis of variance (29).

Results

Proportion of circulating T lymphocytes is higher in commercially raised chickens

Overall, the proportion of circulating T cells was higher in the commercially raised WLH chickens than in laboratory-raised outbred WLH chickens (21–25) (Table II). Figure 1 shows a typical flow

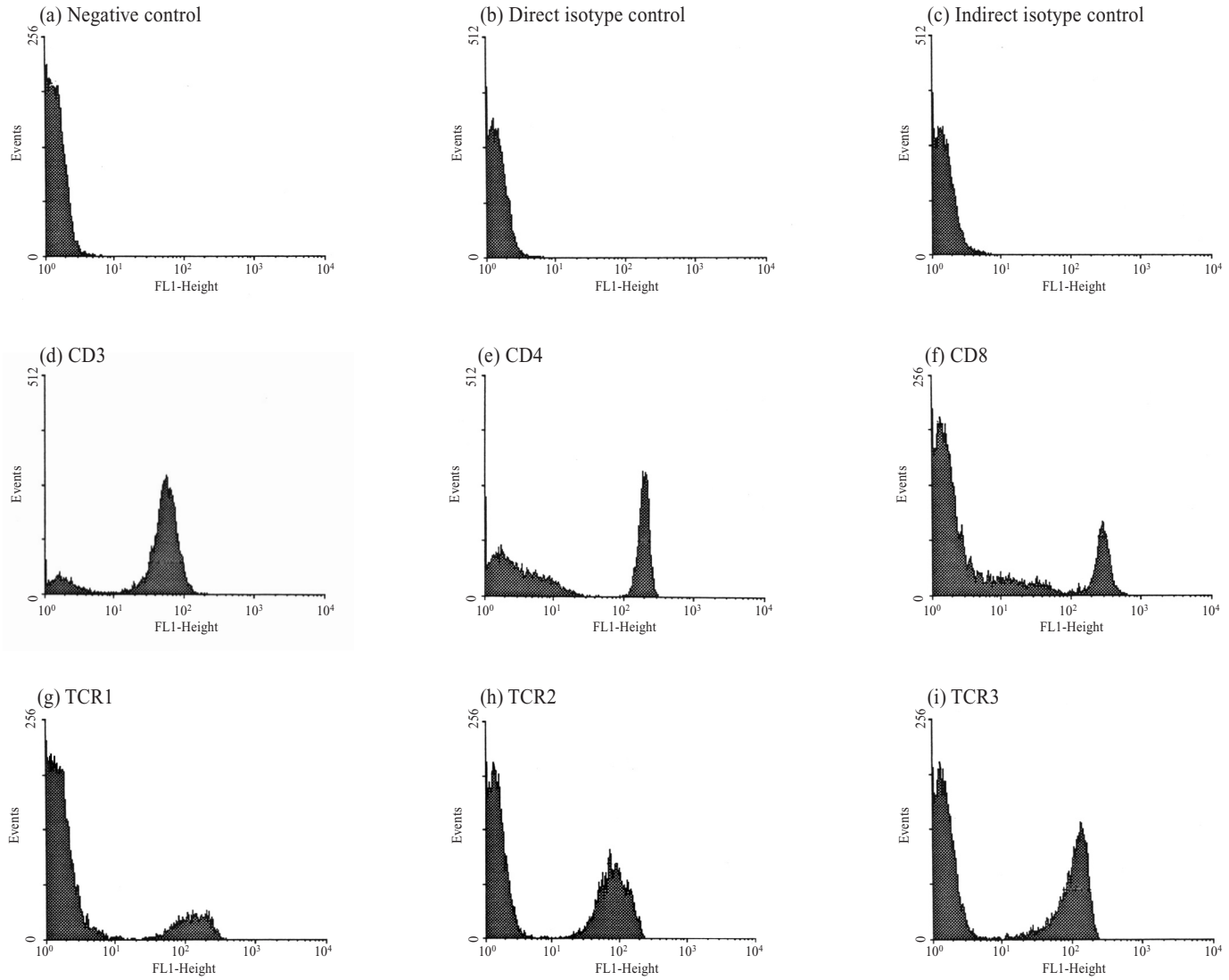

Figure 1. Typical flow cytometry single-color histograms of chicken lymphocytes immunostained for various surface antigens. The negative controls and test antigens were as follows: (a) unlabeled cells; (b) direct isotype control, murine IgG1 coupled to fluorescein isothiocyanate (FITC); (c) indirect isotype control, murine IgG1 coupled to biotin and further labeled with FITC-conjugated avidin; (d) mouse antibody against chicken CD3 coupled to biotin and indirectly labeled with FITC-conjugated avidin; (e) mouse antibody against chicken CD4 coupled to FITC; (f) mouse antibody against chicken CD8 coupled to FITC; (g) mouse antibody against chicken TCR1 coupled to FITC; (h) mouse antibody against chicken TCR2 coupled to FITC; and (i) mouse antibody against chicken TCR3 coupled to biotin and indirectly labeled with FITC-conjugated avidin.

cytometry histogram of lymphocytes labeled for each of the surface antigens. When compared with published values for laboratory-raised outbred WLH chickens (25), the proportion of peripheral blood $CD3^+$ lymphocytes (panel d) was higher in both of the commercially raised hybrids (Table II) and decreased from the 9-wk-old to the 79- or 80-wk-old chickens ($P < 0.0001$). However, in the Dekalb Delta chickens a large decline was evident in the young adults and persisted through adulthood, whereas in the H&N chickens the decrease was more gradual.

Proportion of circulating $CD4^+$ T lymphocytes is higher in commercially raised chickens

The proportion of peripheral blood $CD4^+$ lymphocyte (Figure 1e) was consistently higher in both hybrids, irrespective of age group, than in the outbred WLH chickens (25) (Table II). In the Dekalb Delta hybrid the proportion decreased from 9 to 25 wk of age but then increased by 80 wk, to a value higher than at 9 wk ($P < 0.0001$). By contrast, no significant differences existed in the H&N chickens among the 3 age groups. The increased $CD4^+$ T cell levels in the commercially raised hybrids are consistent with the greater total T cell population.

Proportion of circulating $CD8^+$ T lymphocytes is higher in commercially raised chickens

Consistent with the increased $CD3^+$ levels, the proportion of peripheral blood $CD8^+$ lymphocytes (Figure 1f) was higher in both hybrids of all age groups (Table II) than in outbred WLH chickens (25). The proportion declined from juvenile to adult age in both the Dekalb Delta chickens ($P < 0.005$) and the H&N chickens ($P < 0.0001$).

The CD8 antigen expression varied on the $CD3^+$ T cells, leading to identification of $CD8^{dim+}$ and $CD8^{bright+}$ subpopulations (Figure 2). A remarkable decline in the $CD8^{bright+}$ cells (Figures 1f and 2) in both the Dekalb Delta chickens ($P < 0.0001$) and the H&N chickens ($P < 0.005$) (Table II) correlated with a similar decrease in $CD4^+$ and $CD3^+$ lymphocytes. No significant differences existed in circulating $CD8^{dim+}$ lymphocytes among the age groups for either hybrid. Further, no significant differences in the $CD8^{dim+}$:$CD8^{bright+}$ ratio (Table II) were evident in either hybrid. Overall, these observations suggested a higher proportion of peripheral blood $CD8^+$ T cells in commercially raised chicken hybrids than in outbred WLH chickens.

Proportion of circulating $TCR1^+$ lymphocytes decreases with age

A significantly lower proportion of $TCR1^+$ ($\gamma\delta$) T lymphocytes (Table II, Figure 1g) was noted in the adult chickens (Dekalb Delta, 13.8%; H&N, 19.7%; $P < 0.0001$) when compared with the juvenile chickens (Dekalb Delta, 22.9%; H&N, 28.1%) and the young-adult chickens (Dekalb Delta, 28.9%; H&N, 28.3%). Such a decline, in addition to considering the reference values from laboratory-raised outbred WLH chickens, suggests that the phenomenon is age-related.

Proportion of circulating $TCR2^+$ lymphocytes is increased in commercially raised chickens

The proportion of $TCR2^+$ ($V\beta1$) T cells (Figure 1h) was higher in the Dekalb Delta and H&N hybrids when compared with outbred WLH chickens (Table II). The proportion was significantly lower ($P < 0.0001$ and < 0.005 in the 2 hybrids, respectively) in the 25-wk-old chickens (Dekalb Delta, 43.9%; H&N, 50.5%) than in the 9-wk-old chickens (Dekalb Delta, 54.1%; H&N, 57.1%).

Proportions of circulating $TCR3^+$ lymphocytes are comparable in the juvenile and adult commercially raised chickens

No significant differences existed in $TCR3^+$ ($V\beta2$) lymphocyte levels (Figure 1i) in adult H&N chickens (11.3%) compared with juvenile (10.9%) or young-adult (10.7%) H&N chickens. However, in the Dekalb Delta hybrid the young adults had significantly lower proportions ($P < 0.001$) of $TCR3^+$ T cells (12.6%) than did the other age groups.

The CD4:CD8 ratio is decreased in commercially raised chickens

A remarkably lower CD4:CD8 ratio was noted in all age groups of the 2 commercial hybrids in comparison with the ratio for unimmunized laboratory-raised outbred WLH chickens (Table II). Further, the ratio was significantly higher in the adult hybrids than in the young-adult hybrids: Dekalb Delta, 1.8 ± 0.5 versus 1.3 ± 0.3 ($P < 0.0001$); H&N, 1.9 ± 0.6 versus 1.3 ± 0.3 ($P < 0.005$). These observations suggest that genetic selection, intense immunization, and other environmental factors involved in commercial poultry operations significantly modulate T lymphocyte subpopulations to affect the CD4:CD8 ratio, a measure of immunocompetence.

Discussion

Little is known about the immunologic status of apparently healthy commercially raised layer chicken hybrids that undergo intense immunization for disease prevention and genetic selection for enhanced production traits. The peripheral blood lymphocyte populations are known to be under genetic control (1,8–11) and thus provide an important biomarker for evaluating immunocompetence. We compared values for these populations in healthy commercially raised chicken hybrids with established reference values for unimmunized laboratory-raised outbred WLH chicken (25) to evaluate immunocompetence. It could be argued that the outbred chickens may have genetically diverged over time, but such a comparison is necessitated under field conditions, because unimmunized commercial chickens are unlikely to be available.

This study investigated age-related proportions of peripheral blood T lymphocyte subpopulations in apparently healthy commercially raised WLH chicken hybrids (Dekalb Delta and H&N), with their specific immunization and management protocols. The experiments demonstrated that the proportions were greater in the commercially raised hybrids than in laboratory-raised outbred WLH chickens (25), the divergence being evident in the $CD4^+$, $CD8^+$, and $TCR2^+$ T cell subpopulations. The CD4:CD8 ratio was much lower in the commercially raised chickens, indicating reduced immunocompetence.

Although the various T cell population levels observed in the 2 chicken hybrids were, in general, consistent with those reported from other studies (21,24,25,30,31), the proportion of $CD8^+$ lymphocytes was significantly higher in both of our hybrids than the 10% to 20% reported previously (22). The difference is likely due to inclusion of both $CD8^{dim+}$ and $CD8^{bright+}$ lymphocyte populations in the present study. These 2 subsets of $CD8^+$ T cells identified in WLH chickens seem to reflect homodimeric $\alpha\alpha$ ($CD8^{dim+}$) and heterodimeric $\alpha\beta$ ($CD8^{bright+}$) forms of the CD8 antigen (32,33). The present study extends these observations by providing age-related proportions of circulating $CD8^{dim+}$ and $CD8^{bright+}$ T lymphocyte subsets in commercially raised chickens. Further characterization of the $CD8^{dim+}$ lymphocyte population is required, especially its coexpression with the CD4 molecule, to determine if these lymphocytes represent a subset of $CD3^+$ T cells known to be transiently expressed in healthy and diseased individuals and suggested to be memory T cells with cytolytic ability (34). Chicken intraepithelial lymphocytes are known to acquire CD8 $\alpha\alpha$ homodimers in the gut microenvironment, although these originate from the thymus during early development (35). Whether these lymphocytes circulate in the peripheral blood as a result of bacterial infection (36) needs to be investigated.

Although significantly higher than those in the unimmunized laboratory-raised outbred WLH chickens, the levels of circulating T cells in both the commercially raised chicken hybrids declined with age. There was likely a parallel decrease in circulating B cell levels, compromising the humoral immune capacity of the commercially raised chickens. Both the Dekalb Delta and the H&N hybrids had undergone intense immunization for common poultry pathogens (Table I) until 12 and 16 wk of age, respectively, which would directly influence the proportion of circulating T cells. Since immunizations were done against viruses commonly infecting poultry, the higher proportion of $CD8^+$ T cells in both chicken crosses suggests the

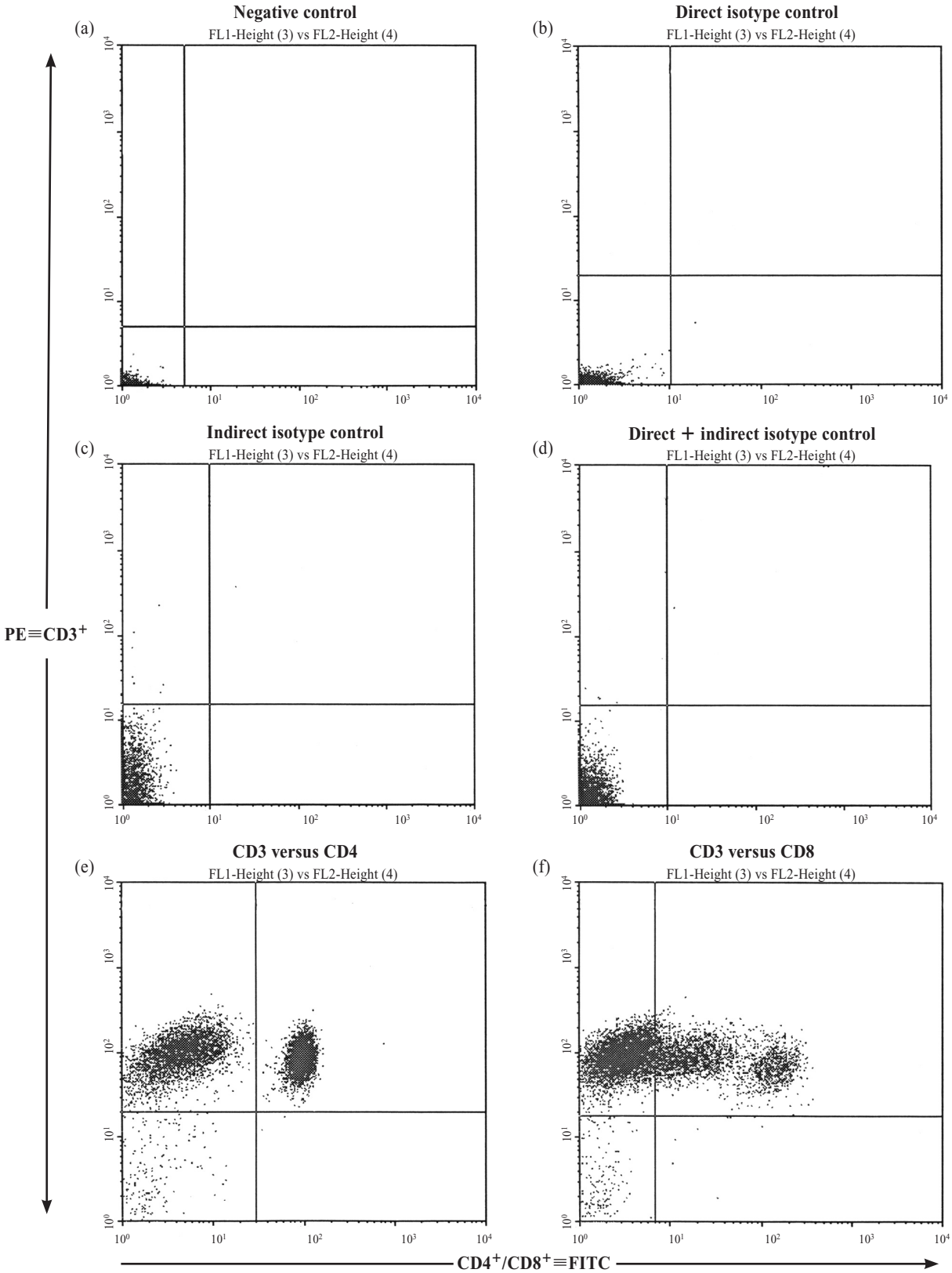

Figure 2. Typical flow cytometry 2-color histograms of chicken lymphocytes immunostained for various surface antigens. The negative controls and test antibodies were as follows: (a) unlabeled cells; (b) direct isotype control, murine IgG1 coupled to FITC; (c) indirect isotype control, murine IgG1 coupled to biotin and further labeled with phycoerythrin (PE)-conjugated avidin; (d) combined direct and indirect isotype control, murine IgG1 coupled to FITC and murine IgG1 coupled to biotin and indirectly labeled with PE-conjugated avidin; (e) mouse antibody against chicken CD3 coupled to biotin and indirectly labeled with PE-conjugated avidin and mouse antibody against chicken CD4 coupled to FITC; and (f) mouse antibody against chicken CD3 coupled to biotin and indirectly labeled with PE-conjugated avidin and mouse antibody against chicken CD8 coupled to FITC.

required elicitation of cell-mediated immunity. Given the ongoing genetic selection in commercial chickens, the contribution of genetic factors that control T cell proportions (1,8–11) is not excluded, apart from those influenced by differing immunization protocols. Regardless of age, the decreased CD4:CD8 ratio in the Dekalb Delta and H&N chickens as compared with the laboratory-raised WLH chickens suggests reduced immunocompetence as a result of commercial operations including intense immunization and genetic and environmental factors. The possibility of confounding methodologic factors is excluded, as we used highly specific reagents and a sensitive analytic technique (flow cytometry). Indeed, the amount and proportion of peripheral blood T cell populations are known to influence immunocompetence responsible for disease susceptibility or resistance (14,15). A higher number or proportion of $CD4^+$ T cells in comparison with $CD8^+$ T cells in blood, thymus, or spleen has immunomodulatory significance, often correlated with wide-ranging immune competence (37,38). A higher number of $CD8^+$ T cells in the joints and peripheral blood has been noted in WLH chickens resistant to *Enterococcus faecalis*, in contrast to the brown layer breed, which is susceptible to the infection (39).

The unusually high proportion of $CD3^+$ T cells and the decreased CD4:CD8 ratio in the commercially raised hybrids compared with outbred WLH chickens reflects decreased immunocompetence, especially humoral immune capacity. Similarly, a low CD4:CD8 ratio in splenocytes from broiler chickens selected for growth performance has been reported (40). The lower CD4:CD8 ratio in the commercial chicken hybrids could be attributed to genetic selection and management factors, especially intense immunization. Such a negative outcome of genetic selection, immunization, and environmental factors on the immune competence of commercial chicken hybrids necessitates development of integrated strategies for balancing multigenic traits that govern immune function and growth or egg-laying capacity. As suggested earlier (7), immune assessment of commercially raised chickens is needed to protect against diseases associated with intensive management practices and to ensure the safe application of therapeutic agents or prophylactic immunization. While evaluating immunocompetence in commercially raised chickens, consideration deserves to be given to the differences observed in the proportions of T cell subsets that diverge from those in unimmunized laboratory-raised outbred WLH chickens.

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